

REMARKS-General

1. Upon review of the original and previously amended specifications and claims, and also in light of the observation of the Examiner noted in the above Office Action, the applicants have further amended the current claims as described above. No new matter has been included. The purpose of the currently amended Claims 1, 11, 12, 22 and 33 is to clearly define and emphasize more directly the distinguishing features of the innovative process for amplifying sense-oriented and full-length versions of mRNAs resulting in novel compositions of sense-oriented full-length RNA amplicons from the Present Invention.

Response to Rejection of Claims 1-3, 7-18, 20, 22, 23, 25, 26 and 29-35 under 35 USC 112, First Paragraph

2. According to the guidance in *In re Wands*, 8 USPQ2d 1400 (CAFC 1988), the applicants respectfully submit that the original claims 1, 11, 12, 22 and 33 are amended to fully match the claimed subject matter of the instant invention to the description of the original specifications and examples, as pursuant to 35USC112, first paragraph.

3. The amended Claim 1 clarifies that the instant invention is a process.

4. The amended Claim 11 corrects a typing error and defines a temperature for denaturation.

5. The amended Claims 12 and 33 described a defined term and clarify the Tth DNA polymerases as previously defined in the Specification as RNA- and DNA-dependent DNA polymerases with reverse transcription activity.

6. The amended Claim 22 corrects a typing error and defines the term of polynucleotide-tailed complementary DNA as described in the Specification.

7. The applicants believe it is not necessary, in view of the Instruction Manual of C. therm. polymerase from Roche Biochemicals (see Reference 1), to describe how the name of C. therm. polymerase was given. Like the given names of Taq and Pwo polymerases, it is general knowledge in the public domain that the term C. therm. was given to a Klenow fragment of the DNA polymerase from *Carboxydotherrmus hydrogenoformans*, not an abbreviation.

8. In response to paragraphs 2-6 of the Examiner's Office Action, the applicants respectively believe that the rejection of Claims 1-3, 7-18, 20, 22, 23, 25, 26 and 29-35 under 35 USC 112, first paragraph, was based on a mistaken interpretation of the Specification of the instant invention. Page 10, last paragraph, describes that the finding of thermostable RNA polymerases may make the procedure of the present invention more convenient although we currently need to add new RNA polymerase in every round of transcription due to the denaturation step. Because the current market only provides heat-labile RNA polymerases, the Specification of Page 10, last paragraph, teaches that one needs to renew the RNA polymerase in every round of transcription during the cycling procedure of the instant invention as shown in the Specification and Example 5. Thus, Page 10, last paragraph, does **NOT** teach the addition of RNA polymerases only once for an infinite number of cycles of amplification. Based on the above limitation, Claims 1 and 22 contain only the **ONE CYCLE** procedure of the instant invention and Claims 2 and 23 include the **FURTHER CYCLING** procedure of Claims 1 and 22 of the instant invention. Further, the respective enzyme activities are claimed in each step of Claims 1 and 22, and are re-claimed in the Claims 2 and 23. Since the applicants did **NOT** suggest the one-time addition of RNA polymerases for an infinite number of cycles of amplification, it is **UNREASONABLE** to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention in this irrelevant direction. As we suggested on Page 10, last paragraph, it is impossible to generate full-length mRNAs by adding RNA polymerases only once for an infinite number of cycles of the present invention, unless a thermostable RNA polymerase is discovered.

9. In response to paragraphs 7-9 of the Examiner's Office Action, the applicants have described and overcome the secondary structures of DNA and RNA at elevated temperatures and use thermostable enzymes widely available in the current market. Unlike a decade ago, the use of thermostable enzymes at elevated temperatures is a general idea in the current public domain used to generate more full-length DNA and RNA (see References 1 and 2). It is also a well-known fact that pre-incubation at 65°C for at least 5 minutes or at 72°C for 2 minutes can greatly reduce the secondary structures as shown in the Examples 2, 4 and 5. Thus, the instant invention **DOES** teach the use of thermostable enzymes at elevated temperatures to improve the problems and difficulties which occurred before. Further, the full-length property of the resulting products of the present invention is ensured by the use of a tailing reaction described in the Examples 2 and 5. Since it is a well-known fact that only the 3'-

protruding and **3'-blunted** ends of full-length cDNAs in the mRNA-cDNA hybrid products of reverse transcription can be tailed by terminal transferase in a magnesium (Mg)-containing reaction as shown in the Examples 2 and 5, the present invention **DOES** provide an improved enzymatic procedure for full-length mRNA amplification.

The applicants also respectively believe that the rejection of Claims 1-3, 7-18, 20, 22, 23, 25, 26 and 29-35 under 35 USC 112, first paragraph, was based on an unreasonable speculation of potential difficulties of the present invention without any practical evidence. The issue of secondary structures has been proven to **NOT** be a statistically significant problem to the present invention. As described in Chapter 12 of a library textbook, *Generation of cDNA Libraries* (see Reference 4), it suggests that the instant invention can cover more than 90% of a whole mRNA population and improves the full-length conformation up to 9 kilobases. In general, the good integrity of total cellular mRNAs appears as a smear between approximately 500 nucleotide bases and 5 kilobases on an electrophoresis gel and is composed of a median size of about 2 to 3 kilobases. It is obvious that the instant invention has maintained the full-length integrity of an amplified mRNA library. This fact is also proven by Ying et al. in their microarray report in the scientific journal of *Biochemical and Biophysical Research Communications* 313:104-109 (see Reference 5), suggesting that the use of the instant invention can maintain up to a 98% correlation coefficient rate of mRNA amplification, which means more than 98% of the original mRNA population has been well preserved in almost the same composition. The composition includes the integrity of length conformation, sequence accuracy and population ratio as for the microarray analysis of 12,258 different genes in Ying's report. These experimental reports have clearly ruled out the speculation of any potential problem of secondary structures and enzymatic fidelity in the present invention. Thus, the issue of secondary structures is **NOT** a proper basis for expanding the uncertainty of potential references in making out an 'undue experimentation' rejection of the Present Invention and Claims. See *In re Gordon*, 221 U.S.P.Q. 1125, 1127 (Fed. Cir. 1984), ("The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification."). In re Laskowski, 10 U.S.P.Q.2d 1397, 1398 (Fed. Cir. 1989), ("Although the Commissioner suggests that [the structure in the primary prior art reference] could readily be modified to form the [claimed] structure, "[t]he mere fact that the prior art could be modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.")

10. In response to paragraph 10 of the Examiner's Office Action, the applicants respectively believe that the rejection of Claims 1-3, 7-18, 20, 22, 23, 25, 26 and 29-35 under 35 USC 112, first paragraph, was also based on a misunderstanding of the Specification of the Present Invention. The examiner described the use of eukaryotic type II RNA polymerases, transcription factors and TATA box for mRNA generation in view of US Patent Publication 2003/0087275. However, the currently available in-vitro transcription is all based on the use of prokaryotic RNA polymerases, such as T7, T3 and SP6 bacteriophage polymerases. Unlike eukaryotic type II RNA polymerases, the bacteriophage RNA polymerases are single polypeptides of about 98 kD containing all the activities necessary for specific initiation, elongation and termination of RNA synthesis (see Reference 3). They recognize RNA promoters containing a phage-specific TATA box as shown in SEQ ID NO. 1, 3 and 5, which have been described to be incorporated into the amplified cDNA templates for mRNA amplification in the Specification and Examples 2, 4 and 5. No eukaryotic transcription factor is needed for the claimed in-vitro transcription. Thus, the reference of US Patent Publication 2003/0087275 is **NOT** relevant to the present invention.

11. In response to paragraph 11 of the Examiner's Office Action, the Inventors agree that there is no perfect enzyme with 100% fidelity. However, the description of US Patent Publication 2003/0040099 obviously exaggerates the effect of enzymatic fidelity bias without solid experimental evidence. Based on current enzyme isolation technologies, the availability of pure and high-fidelity enzymes in the current market has significantly minimized this issue. Numerous commercially available enzymes possess highly compatible fidelity as the native proofreading enzymes (see Reference 2). Further, in view of Chapter 12 of a library textbook, Generation of cDNA Libraries (see Reference 4) and Ying's microarray report in the journal of Biochemical and Biophysical Research Communications 313:104-109 (see Reference 5), both experimental reports suggest that the present invention can maintain up to 98% of the original mRNA composition, including the integrity of length conformation, sequence accuracy and population ratio. The resulting 2% bias after two cycles of amplification is actually **NOT** statistically significant. Because the basic conditions and procedures are mostly different between the US Patent Publication 2003/0040099 and the present invention, it is difficult to compare these two inventions for respective fidelity improvement. Thus, the reference of US Patent Publication 2003/0040099 to the present invention is **NOT** appropriate.

12. In response to paragraph 12 of the Examiner's Office Action, the applicants have described and overcome the amplification efficiency of DNA-dependent and RNA-dependent DNA polymerases at elevated temperatures and use thermostable enzymes (see References 1 and 2). Unlike the description of low amplification efficiency of conventional heat-labile enzymes in the US Patent 6,303,306 B1, the use of thermostable enzymes at elevated temperatures is a general idea in current public domain to increase amplification efficiency. As shown in Examples 1-5, the instant invention **DOES** teach the use of thermostable enzymes at elevated temperatures to improve the amplification efficiency of the present invention. By running reactions at about 65-72°C temperatures, most secondary structures can be eliminated and highly specific primer annealing and extension can be achieved. However, the current RNA polymerases for in-vitro transcription are all heat-labile. Thus, Page 10, last paragraph, teaches that one needs to renew the RNA polymerase in every round of transcription during the cycling procedure of the instant invention, and the finding of thermostable RNA polymerases may make the procedure of the present invention more convenient. On the other hand, Jia et al. have reported in the journal of Biochemistry 36:4223-4232 that the conformational change of T7 RNA polymerase plays a role in the proper alignment of the initiating and elongating NTPs for efficient phosphodiester bond formation and in maintaining the fidelity of RNA synthesis (see Reference 3). This suggests that the fidelity and efficiency of T7 RNA polymerase is most likely much better than the description of US Patent 6,303,306 B1. Further, a greater than 250-fold amplification rate per cycle was found in the present invention as reported in Chapter 12 of the textbook, Generation of cDNA Libraries (see Reference 4). This finding suggests that the amplification efficiency of three cycles of the present invention is equal to the amplification rate of thirty cycles of PCR, providing a more than fifteen million fold amplification rate. Again, it is clear that the prediction of US Patent 6,303,306 B1 does **NOT** fit the fact of actually experimental results. Due to the uncertainty of enzyme efficiency and the lack of experimental support in the descriptions of US Patent 6,303,306 B1 and US Patent Publication 2003/0040099, these references, in view of the above experimental results, to the present invention is suggested to be **NOT** appropriate.

13. In response to paragraph 13 of the Examiner's Office Action, the applicants respectfully agree in part with the Examiner that the currently available DNA-dependent RNA polymerases may not incorporate fluorescently labeled NTPs during RNA synthesis as described in the US Patent 6,140,053. Because the proofreading activity

of most RNA polymerases, it is likely to be a possible event. However, we did and do **NOT** claim for the use of fluorescently labeled NTPs in the present invention. The description of the use of fluorescently labeled NTPs is **NOT** relevant to the present invention.

14. In response to paragraph 14 of the Examiner's Office Action, the applicants respectfully believe that the rejection of Claims 1-3, 7-18, 20, 22, 23, 25, 26 and 29-35 under 35 USC 112, first paragraph, was further based on a misunderstanding of the instant invention. The present invention is **NOT** a PCR-based procedure, thus the comparison between RT-PCR and the present invention is **NOT** appropriate. The present invention improves upon the problems and difficulties of RT-PCR using transcriptional amplification instead of PCR amplification. Since the transcriptional amplification generates RNA products and is not significantly affected by the secondary structures of RNAs, it has been proven that the products of the instant invention can cover more than 90% of a whole mRNA population and improve the full-length conformation up to 9 kilobases as described in Chapter12 of the textbook, Generation of cDNA Libraries (see Reference 4). The use of thermostable enzymes at elevated reaction temperatures also improves the results of the present invention as described in Examples 1-5. Thus, there is **NO** significant problem of secondary structures in the present invention.

15. In response to paragraph 15 of the Examiner's Office Action, the reference of enzymatic coupling efficiency and accuracy of the iterative methods described in US Patent 5,858,671 is **NOT** relevant to the present invention. The iterative method refers to a procedure of iterative cycles of adaptor ligation and IIS cutting to create new single-stranded DNA templates for sequencing by template-directed polymerization. Both adaptors and DNA templates are still largely maintained in their own double-stranded conformation during annealing. In one embodiment of the US Patent 5,858,671, the DNA products of PCR can be used as DNA templates in the iterative method, thus, the 99% coupling efficiency is **NOT** meant to be the primer annealing efficiency of PCR. The enzymatic coupling efficiency of the iterative methods is based on the annealing between the single-strand overhangs of the adaptors and the IIS-cleaved single-stranded overhangs of the DNA templates. Since the IIS cutting only provides a maximal 9-base single-strand DNA region for annealing, the coupling efficiency is estimated to be poor at such a low annealing temperature, approximately less than 27°C. In contrast, the present invention is based on the annealing of two long matched

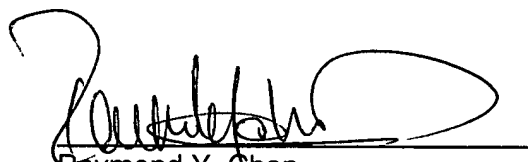
single-stranded regions between primers and denatured templates at elevated temperatures above 54°C. Both primers and templates are single-stranded. It is clear that the estimation of enzymatic coupling efficiency of the iterative methods described in US Patent 5,858,671 is **irrelevant** to either PCR or the present invention.

16. In view of above, the referenced prior arts, as speculated by the Examiner, are based on an uncertain description and irrelevant conditions to the instant invention. There is no proper basis for such a speculation. Therefore, the basis for decision of claim rejection to Claims 1-3, 7-18, 20, 22, 23, 25, 26 and 29-35 under 35 U.S.C. 112, first paragraph, is unfound.

17. The applicants believe that for all of the foregoing reasons, all of the Claims 1-3, 7-18, 20, 22, 23, 25, 26 and 29-35 are in condition for allowance and such action is respectfully requested.

18. Should the Examiner believe that anything further is needed in order to place the application in condition for allowance, he is requested to contact the undersigned at the telephone number listed below.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Raymond Y. Chan', is written over a horizontal line.

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